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New records of fungal pathogens of invertebrates from endemic pine forests in Mexico

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Abstract

We report fungal pathogens of invertebrates (FPI) (Entomophthorales, Hypocreales, and Orbiliales) from roots of an endemic Mexican pine, *Pinus greggii* Engelm. ex Parl., at four primary montane forests using next-generation sequencing. We found twenty-nine OTUs from 18 genera of FPI associated to the roots of *P. greggii*. New records for Mexico are: *Beauveria felina* (DC.) J.W.Carmich., *Dactylella mammillata* S.M. Dixon, *Dactylella ramosa* Matsushima, *Drechslerella brochopaga* (Drechsler) M. Scholler, Hagedorn & A. Rubner, *Hirsutella minnesotensis* Chen, Liu & Chen, *Leptobacillium leptobactrum* (W.Gams) Zare & W.Gams, *Metapochonia variabilis* Z.F.Zhang, F.Liu & L.Cai, *Monacrosporium leptosporum* (Drechsler) A. Rubner, and *Simplicillium aogashimaense* Nonaka, Kaifuchi & Masuma. A largely unknown array of fungal pathogens of invertebrates are likely to be found in Mexican forests. This work facilitates future analyses of fungal diversity in these primary forests, as well as basic and applied research in biological control.

Keywords

Biological control, Ecdysozoa, entomopathogens, pine forest

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Introduction

Soil, including the rhizoplane and roots, is the natural habitat and reservoir for many fungal pathogens of invertebrates (FPI). These fungi play a crucial role in the population dynamics of their hosts and are important in biological control and sustainable management of invertebrate pests (Toepfer et al. 2010). Their hosts include many ecdysozoans: arthropods, nematodes, and tardigrades. Some FPI are endophytic and participate in nitrogen transfer from insects to plants (Behie et al. 2012). Forests are reservoirs of FPI (Sánchez-Peña et al. 2011). There is little information on FPI from natural pine forests in Mexico. We profiled the community structure of FPI in a Mexican pine, *Pinus greggii* Engelm. ex Parl., forest and indicate the systematic and trophic position of

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the fungi found. This information derives from a project on fungal associates of *P. greggii* roots in Mexico (Casique-Valdés et al. 2020); herein, we address specific information on FPI associated with pine roots.

Methods

We collected *Pinus greggii* roots on 8–24 January 2015 from natural forest stands within the Northern Sierra Madre Oriental pine-oak forest biome of México (Fig. 1). The climate there is BSk: temperate and arid, with an average annual temperature 16–18 °C and an average annual precipitation of 350–400 mm (García 2004). We sampled stands in the state of Nuevo León, at La Tapona, Galeana (024°43′39.90″N, 100°06′44.33″W, 2080 m a.s.l.); and in the state of Coahuila, at Cañón de los Caballos, Saltillo (025°14′47.13″N, 100°54′46.61″W, 2620 m a.s.l.), at Cuauhtémoc, Saltillo (025°17′17″N, 100°55′06″W, 2423 m a.s.l.), and at Jamé, Arteaga (025°23′00″N, 100°30′60″W, 2510 m a.s.l.) (Fig. 1). Roots under the canopy of 20 *P. greggii* trees (separated by >10 m) were sampled randomly over 1 ha/stand. Two root samples/tree were taken by digging 15 cm deep at 1.3 m from the trunk on opposite sides. Approximately 5–10 g of secondary roots per tree were collected from each stand; these samples were washed with tap water for 5 min to disintegrate soil clods, and small amounts of residual soil remained. Blotted roots were stored at −80 °C prior to DNA isolation. The roots from 20 trees (ca. 100 g) were processed. They were pooled and frozen in liquid nitrogen and ground with mortar and pestle (Fig. 1). DNA was extracted from 1 g of the resulting powder using an EZ-10® Spin Column extraction kit (Biobasic Inc., Markham, Ontario, Canada) (Fig. 2). One positive (soil DNA) and one negative control (sterile distilled water) were processed along with DNA samples. PCR was carried out in 25 µL, including 2 µL of 2 ng/µL genomic DNA, 4 µL of 4× HOT FIREPol® DNA Polymerase (Solis Biodyne, Tartu, Estonia), and 0.5 µL of primers (ITS3 primer and ITS4ngsuni variants at 20 uM) designed by Tedersoo et al. (2014) and Tedersoo and Lindahl (2016). The PCR volume was completed with nuclease-free water. PCR conditions were initial denaturation at 95 °C for 15 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 60 s, and final elongation at 72 °C for 600 s

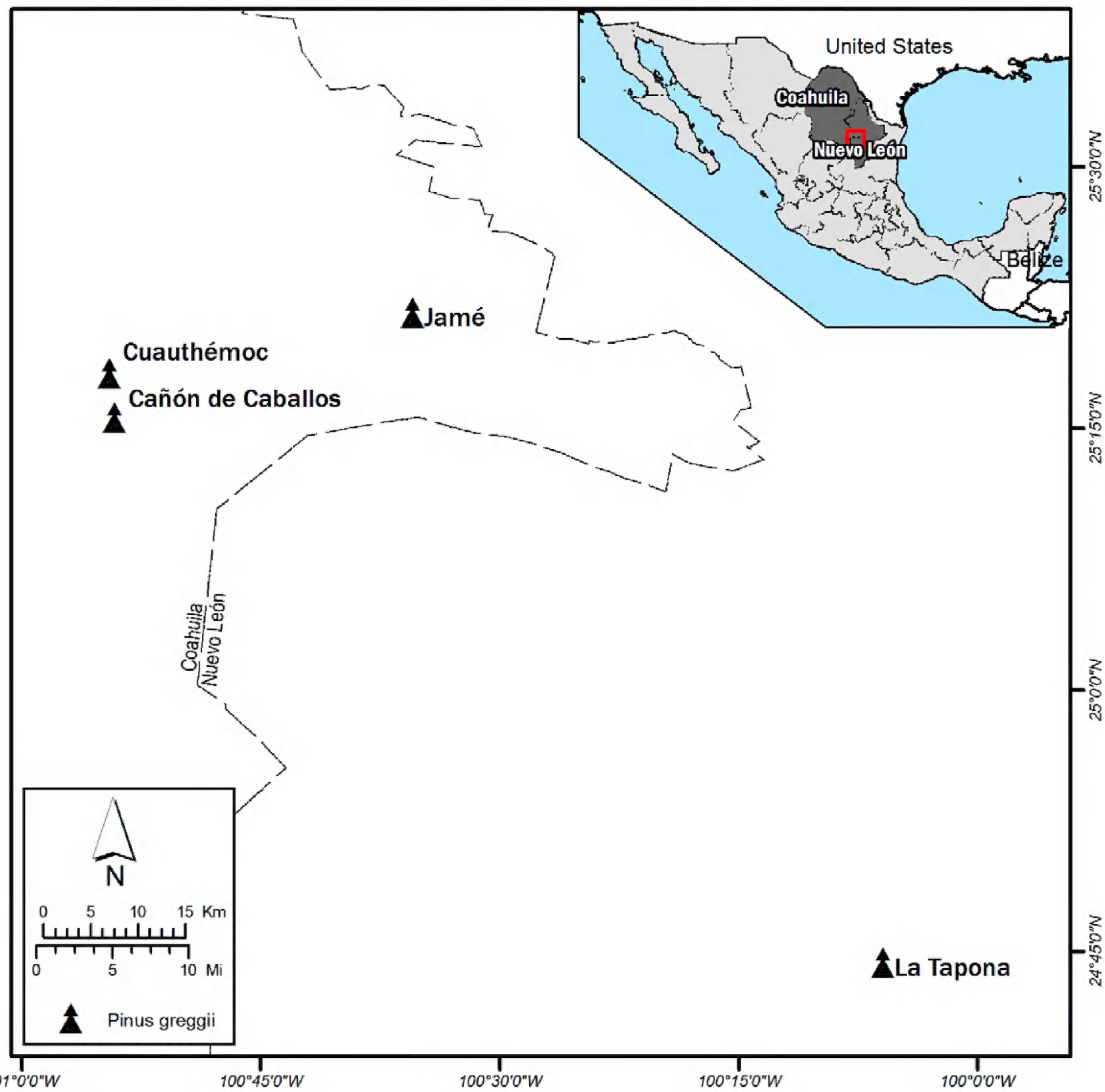

Figure 1. Sampled stands of *Pinus greggii.*

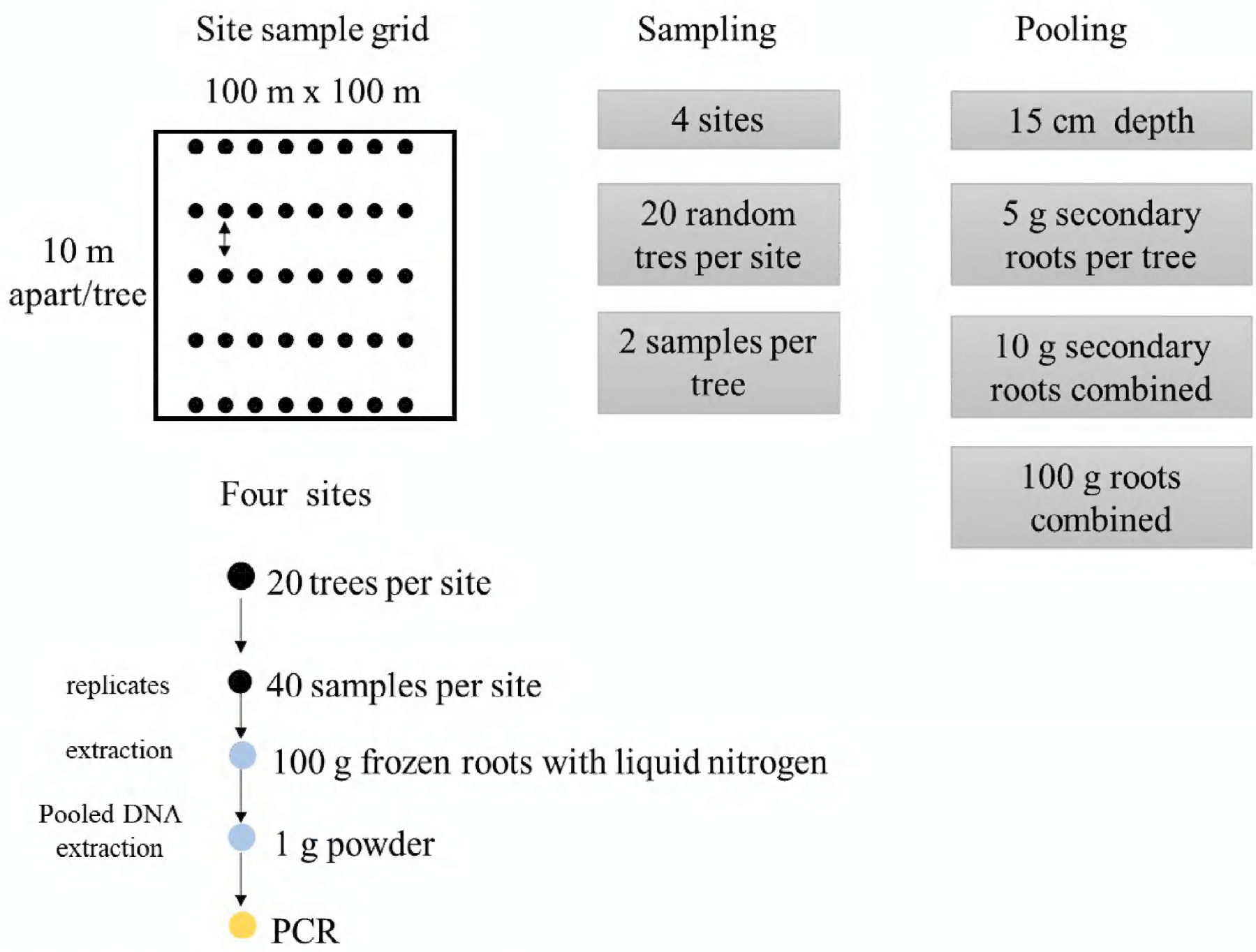

Figure 2. Overview of sample collection and processing.

(Casique-Valdés et al. 2020). Generated amplicons were sequenced (Illumina) using a MiSeq (2×300 bp) instrument. Raw paired-end Illumina data were processed using PipeCraft v. 1.0 (Anslan et al. 2017; Casique-Valdes et al. 2020). Filtered sequences were clustered to OTUs (97% sequence similarity threshold) and Blastn (Camacho et al. 2009) searched (UNITE v. 7.0 reference dataset; Abarenkov et al. 2010) for taxonomic annotation of the OTUs. From the list of OTUs, those with a blastn e-value more than $10e^{-100}$ were eliminated for more confident assignment. Sequences were filtered and manually selected among relevant fungal taxa (i.e., orders Orbiliales, Hypocreales, and Entomophthorales) (Tedersoo et al. 2020) after a BLAST search was performed on GenBank (97% similarity cutoff).

To identify new reports of species for Mexico we searched in biological databases as well as publications including the 2018 "protochecklist" of North American nonlichenized fungi (Bates and Miller 2018), Global Fungi (Robert et al. 2013), the Mycobank database (Větrovský et al. 2020), and the Global Biodiversity Information Facility (GBIF 2021). Maps were created in Arcmap v. 10.8.1 (ESRI 2020).

Results

We found a total of 2081 sequences in 29 OTUs from 18 genera of FPI associated to the roots of *Pinus greggii*. Sequences were accessed in the GenBank database and include both nematophagous and entomopathogenic fungi. A remarkable diversity (15 species) of nematophagous fungi was found; these primarily belong to the family Orbiliaceae (Ascomycota, Orbiliomycetes, Orbiliales) (Table 1) and include the genera *Arthrobotrys*, *Dactylella*, *Dactylellina*, *Drechslerella*, and *Monacrosporium* (Niu and Zhang 2011). Among nematophagous fungi in the Hypocreales, the genera *Pochonia* (Clavicipitaceae), *Metapochonia*, and *Leptobacillium* (Cordycipitaceae), and *Haptocillium*, *Hirsutella*, and *Purpureocillium* (Ophiocordycipitaceae) were recorded (Chen et al. 2000; Evans and Kirk 2017; Hajji et al. 2017).

The entomopathogenic fungi found are in the Ascomycota (Hypocreales): the genera *Beauveria* and *Marquandomyces* (Clavicipitaceae); *Isaria*, *Lecanicillium* and *Simplicillium* (Cordycipitaceae); *Purpureocillium* (also nematophagous) and *Tolypocladium* (*Elaphocordyceps*) (Ophiocordycipitaceae) (Humber 2012; Genier et al 2016; Chen et al. 2019; Jauregui et al. 2020). In the Zoopagomycota: Entomophthorales, the entomopathogen *Zoophthora radicans* (Brefeld) Batko was detected (Torres-Acosta et al. 2016).

Stands and the OTUs found were Jame (17), Cuauhtémoc (16), Tapona (13), and Caballos (2). Table 1 shows new records of FPI for the country (marked with an asterisk) and the sequences found at each locality.

Table 1. OTUs of invertebrate-pathogenic fungi associated with roots from *Pinus greggii* forests in northeastern Mexico.

OTU species name	Lifestyle	GenBank match	%ID	% cover	GenBank sequence	Number of sequences per OTU at each forest stand						Total sequences
						Jame	Cuauh.	Tapona	Cañón Caballos	PC	NC	
Arthrobotrys conoides	N	AF106534.1	97.3	97	MK412796.1	0	0	4	0	0	0	4
Beauveria bassiana	A	MT533246.1	99.7	97	MK412808.1	0	0	10	0	0	0	10
**Beauveria felina*	A?	MH483812.1	97.2	100	MK412812.1	0	13	3	0	0	0	16
Beauveria pseudobassiana	A	MT279264.1	100	100	MZ816951.1	9	9	16	0	0	0	34
Cordyceps farinosa (= *Isaria farinosa*)	A	KT224841.1	100	100	MK412817.1	1619	29	0	0	2	0	1646
**Dactylella mammillata*	N	KT215290.1	100	100	MK412824.1	4	7	0	0	2	0	11
**Dactylella ramosa*	N	DQ494361.1	98	98	MZ618649.1	0	0	25	0	0	0	25
Dactylellina drechsleri	N	MH179742.1	98.4	100	MK412829.1	0	5	0	0	0	0	5
Dactylellina ellipsospora	N	KT215202.1	100	100	MK412828.1	0	21	0	0	0	0	21
**Drechslerella brochopaga* (=*Arthrobotrys brochopaga*)	N	EF445987.1	98.7	100	MK412831.1	2	4	0	2	0	0	8
Drechslerella dactyloides	N	MH862282.1	99.3	97	MK412830.1	0	0	4	0	0	0	4
Haptocillium sp.	N	AJ292417.1	99	96	MK412807.1	9	3	0	0	0	0	12
Haptocillium balanoides	N	EF546660.1	98.6	99	MK412806.1	0	2	0	0	0	0	2
**Hirsutella minnesotensis*	N	KJ524682.1	99.7	97	MK412832.1	0	0	2	0	0	0	2
Lecanicillium aphanocladii	A	MN944448.1	99.7	97	MK412802.1	0	0	5	0	0	0	5
Lecanicillium fungicola	A, M	MT176478.1	100	96	MK412803.1	0	3	0	0	0	0	3
Lecanicillium psalliotae	A, M	MK164208.1	100	100	MK412820.1	4	0	7	0	0	0	11
Lecanicillium psalliotae	A, M	KJ191568.1	98.4	100	MK412819.1	7	0	0	0	0	0	7
Lecanicillium sp	A	MT361811.1	99	100	MK412801.1	15	9	0	0	0	0	24
**Leptobacillium leptobactrum*	N	JQ782652.1	100	100	MZ606659.1	5	0	0	0	0	0	5
**Metapochonia variabilis*	N	KU746684.1	100	100	MK412833.1	36	3	30	0	0	0	69
Marquandomyces marquandii	A?	MT453297.1	99.4	97	MK412838.1	0	0	2	0	0	0	2
**Monacrosporium leptosporum* (=*Dactylella leptospora*)	N	AY773466.1	96.2	100	MK412835.1	3	11	2	0	0	0	16
Pochonia chlamydosporia	N	GQ369959.1	100	97	MK412834.1	12	60	0	3	0	0	75
Purpureocillium lilacinum	A, N	EU553283.1	99	92	MK412837.1	2	0	6	0	0	0	8
**Simplicillium aogashimaense*	A	MN518399.1	97.2	100	MK412798.1	4	0	0	0	0	0	4
Simplicillium cylindrosporum	A	LC228053.1	100	100	MK412813.1	24	2	0	0	0	0	26
Tolypocladium sp. (= *Elaphocordyceps*)	A, M?	KC237381.1	99.6	100	MK412821.1	4	0	0	0	0	0	4
Zoophthora radicans	A	EF151412.1	99.7	100	MK412822.1	13	9	0	0	0	0	22
OTUs total = 29												
OTUs per stand						17	16	13	2			
Total sequences						1772	190	116	5			2081

* = First record for Mexico; A = pathogen of arthropods; N = pathogen of nematodes; M = mycoparasite; PC = positive control (soil sample); NC = negative control (sterile distilled water).

New records of FPI for Mexico: entomopathogenic fungal species

***Beauveria felina* (DC.) J.W. Carmichael**

New records. MEXICO – **Coahuila** • Arteaga, Jame; 25°23′00″N, 100°30′60″W; 2510 m alt.; 08.I.2015; R. Casique-Valdés leg.; oak-pine forest/roots sample/pooled DNA; Blast ID 1367; GenBank MK412812 (ITS) – **Coahuila** • Saltillo, Cuauhtémoc; 25°17′17″N, 100°55′06″W; 2423 m alt.; 12.I.2015; same collector leg.; pine forest/roots sample/pooled DNA; Blast ID 1367; GenBank MK412812 (ITS) • Cañón de Caballos; 25°14′47.13″N, 100°54′46.61″W; 2620 m alt.; 12.I.2015; same method sample, and collector leg.; pine forest; Blast ID 1367; GenBank MK412812 (ITS). Figure 3.

Identification. Generated amplicons were sequenced (Illumina) using a MiSeq (2×300 bp) instrument. Raw paired-end Illumina data were processed, filtered, and clustered to OTUs and Blastn searched; 97.2% identity with GenBank accession MH483812.1 (16 sequences in total).

***Simplicillium aogashimaense* Nonaka, Kaifuchi & Masuma**

New record. MEXICO – **Coahuila** • Arteaga; 25°23′00″N, 100°30′60″W; 2510 m alt.; 08.I.2015; R. Casique-Valdés leg.; oak-pine forest/roots sample/pooled DNA; Blast ID 2760; GenBank MK412798.1 (ITS). Figure 4.

Identification. Obtained OTU as described above was Blastn searched with 97.2% identity with GenBank MN518399.1 with four sequences.

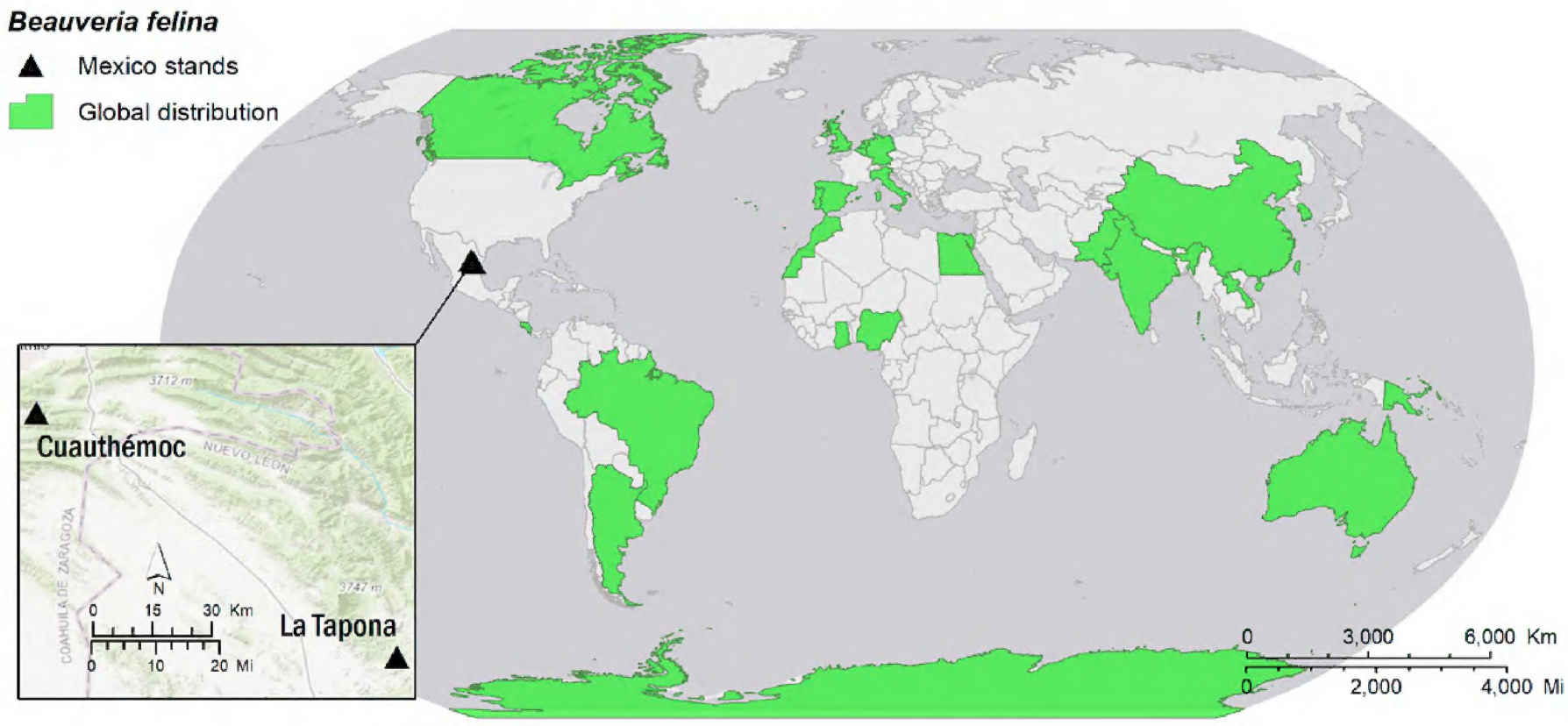

Figure 3. Historical global and new Mexican records of *Beauveria felina*. © 2021 ESRI, Here.

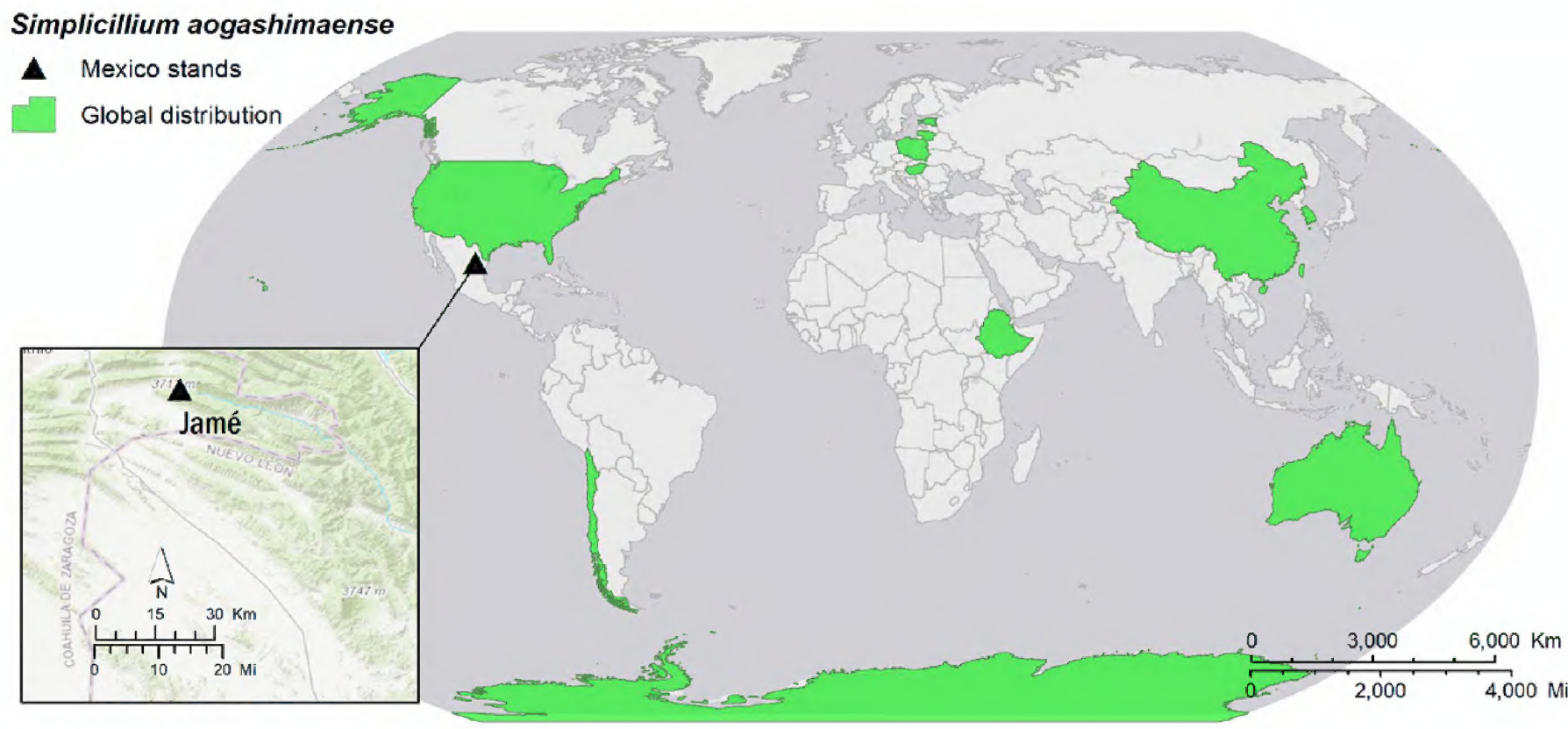

Figure 4. Historical global and new Mexican records of *Simplicillium aogashimaense*. © 2021 ESRI, Here.

New records of FPI for Mexico: nematophagous fungal species

Dactylella mammillata S.M. Dixon

New record. MEXICO – **Coahuila** • Arteaga, Jamé; 25°23′00″N, 100°30′60″W; 2510 m alt.; 08.I.2015; R. Casique-Valdés leg.; oak-pine forest/roots sample/pooled DNA; Blast ID 1507; GenBank MK412824.1 (ITS) • Saltillo, Cuauhtémoc; 25°17′17″N, 100°55′06″W; 2423 m alt.; 12.I.2015; same collector leg.; pine forest/roots sample/pooled DNA; Blast ID 1507; GenBank MK412824.1 (ITS). Figure 5.

Identification. The obtained OTU (Illumina; MiSeq (2×300 bp) instrument) was Blastn searched; 100% identity with GenBank accession KT215290.1 with 11 sequences.

Dactylella ramosa Matsushima

New record. MEXICO- **Nuevo León** • La Tapona; 24°43′39.90″N, 100°06′44.33″W; 2080 m alt.; 24.I.2015; R. Casique-Valdés leg.; pine forest/pooled/roots/pooled DNA; Blast ID 1100; GenBank MZ618649.1 (ITS). Figure 6.

Identification. Generated amplicons were sequenced (Illumina) using a MiSeq (2×300 bp) instrument. Raw paired-end Illumina data were processed and clustered to OTUs; obtained OTU as described above was Blastn searched with 98% identity with GenBank accession DQ494361.1 with 25 sequences.

Drechslerella brochopaga (Drechsler) M. Scholler, Hagedorn & A. Rubner

New records. MEXICO – **Coahuila** • Arteaga, Jame; 25°23′00″N, 100°30′60″W; 2510 m alt.; 08.I.2015; R. Casique-Valdés leg.; oak-pine forest/roots sample/pooled DNA; Blast ID 1950; GenBank MK412831.1 (ITS) • Saltillo, Cuauhtémoc; 25°17′17″N, 100°55′06″W; 2423 m alt.; 12.I.2015; same collector leg.; pine forest/

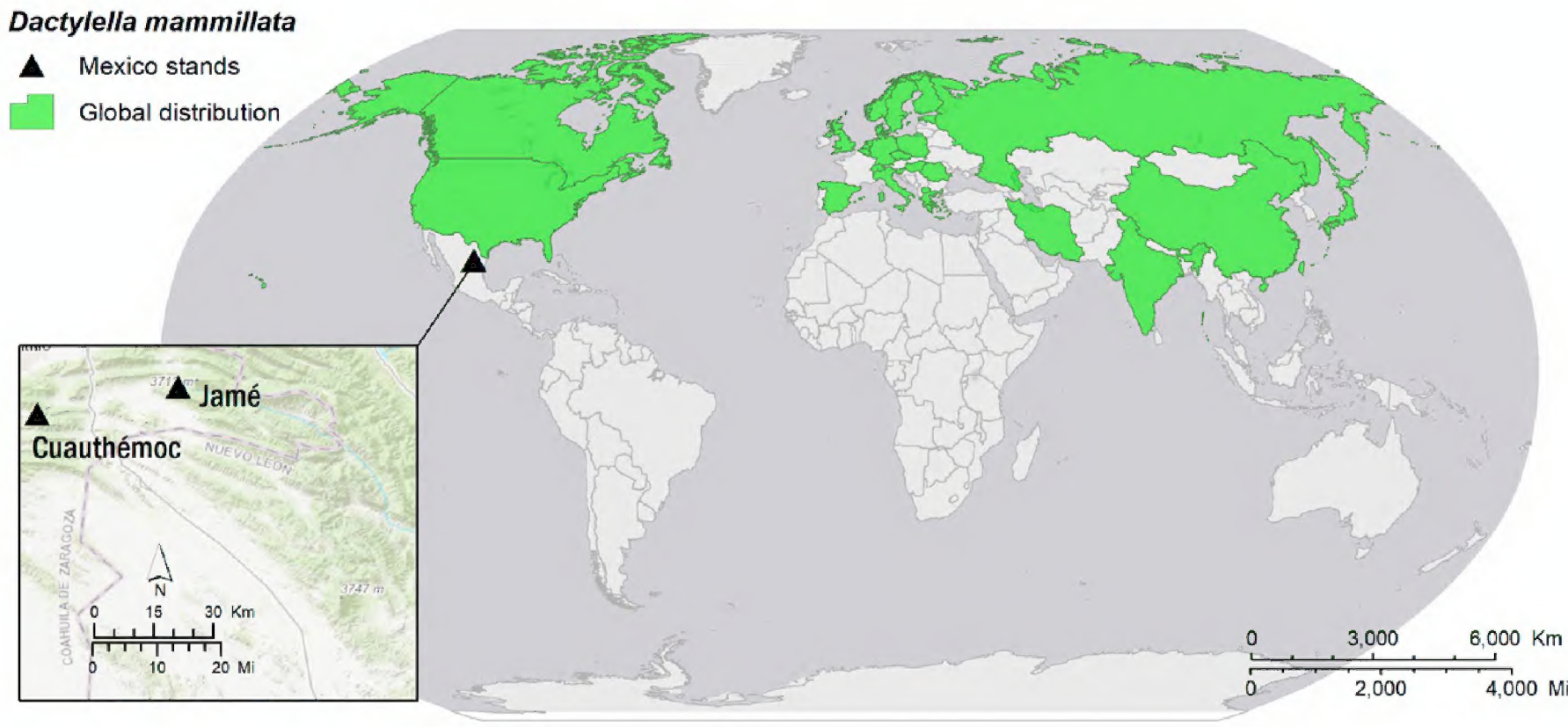

Figure 5. Historical global and new Mexican records of *Dactylella mammillata*. © 2021 ESRI, Here.

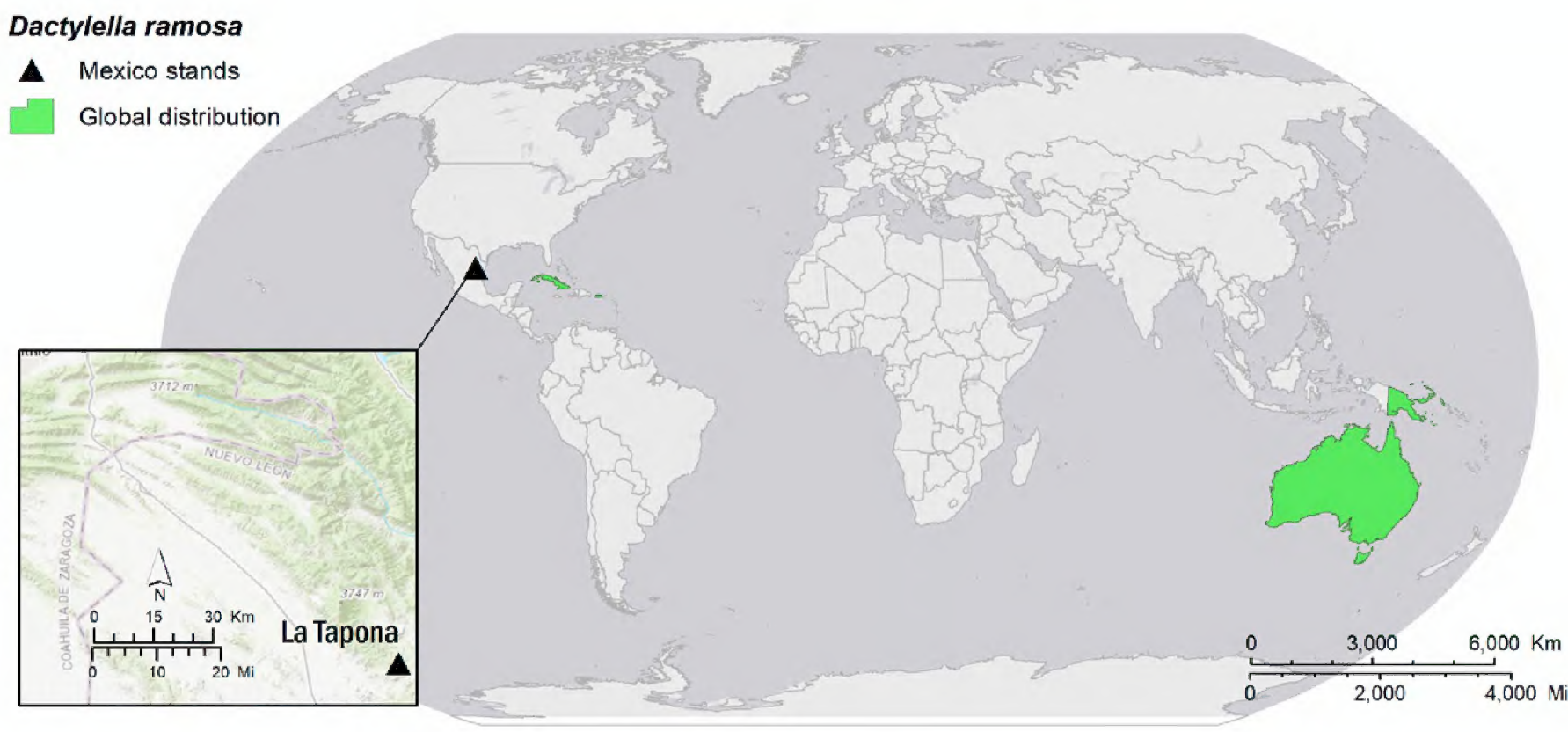

Figure 6. Historical global and new Mexican records of *Dactylella ramosa*. © 2021 ESRI, Here.

roots sample/pooled DNA; Blast ID 1950; GenBank MK412831.1 (ITS). Figure 7.

Identification. Obtained amplicons were sequenced (Illumina) using a MiSeq (2×300 bp) instrument. Clustered OTUs were Blastn searched; 98.7% identity with GenBank accession EF445987.1 (8 sequences in total) was found.

Hirsutella minnesotensis Chen, Liu & Chen

New record. MEXICO – **Nuevo León** • La Tapona; 24°43′ 39.90″N, 100°06′44.33″W; 2080 m alt.; 24.I.2015; R. Casique-Valdés leg.; pine forest/pooled/roots/pooled DNA; Blast ID 3996; GenBank MK412832.1 (ITS). Figure 8.

Identification. Raw paired-end Illumina data were processed and clustered to OTUs. The obtained OTU was Blastn searched with 99.7% identity match with GenBank accession KJ524682.1 with two sequences.

Leptobacillium leptobactrum (W.Gams) Zare & W.Gams

New record. MEXICO – **Coahuila** • Saltillo, Cuauhtémoc; 25°17′17″N, 100°55′06″W; 2423 m alt.; 12.I.2015; R. Casique-Valdés leg.; pine forest/roots sample/pooled DNA; Blast ID 2448; GenBank MZ606659.1 (ITS). Figure 9.

Identification. Generated amplicons were sequenced using a MiSeq (2×300 bp) Illumina instrument. Obtained OTU was Blastn searched with 100% match with GenBank accession JQ782652.1 with five sequences.

Metapochonia variabilis Z.F.Zhang, F.Liu & L.Cai

New records. MEXICO – **Coahuila** • Arteaga, Jame; 25° 23′00″N, 100°30′60″W; 2510 m alt.; 08.I.2015; R. Casique-Valdés leg.; oak-pine forest/roots sample/pooled DNA; Blast ID 645; GenBank MK412833.1 (ITS) • Saltillo, Cuauhtémoc; 25°17′17″N, 100°55′06″W; 2423 m alt.; 12.I.2015; R. Casique-Valdés leg.; pine for-

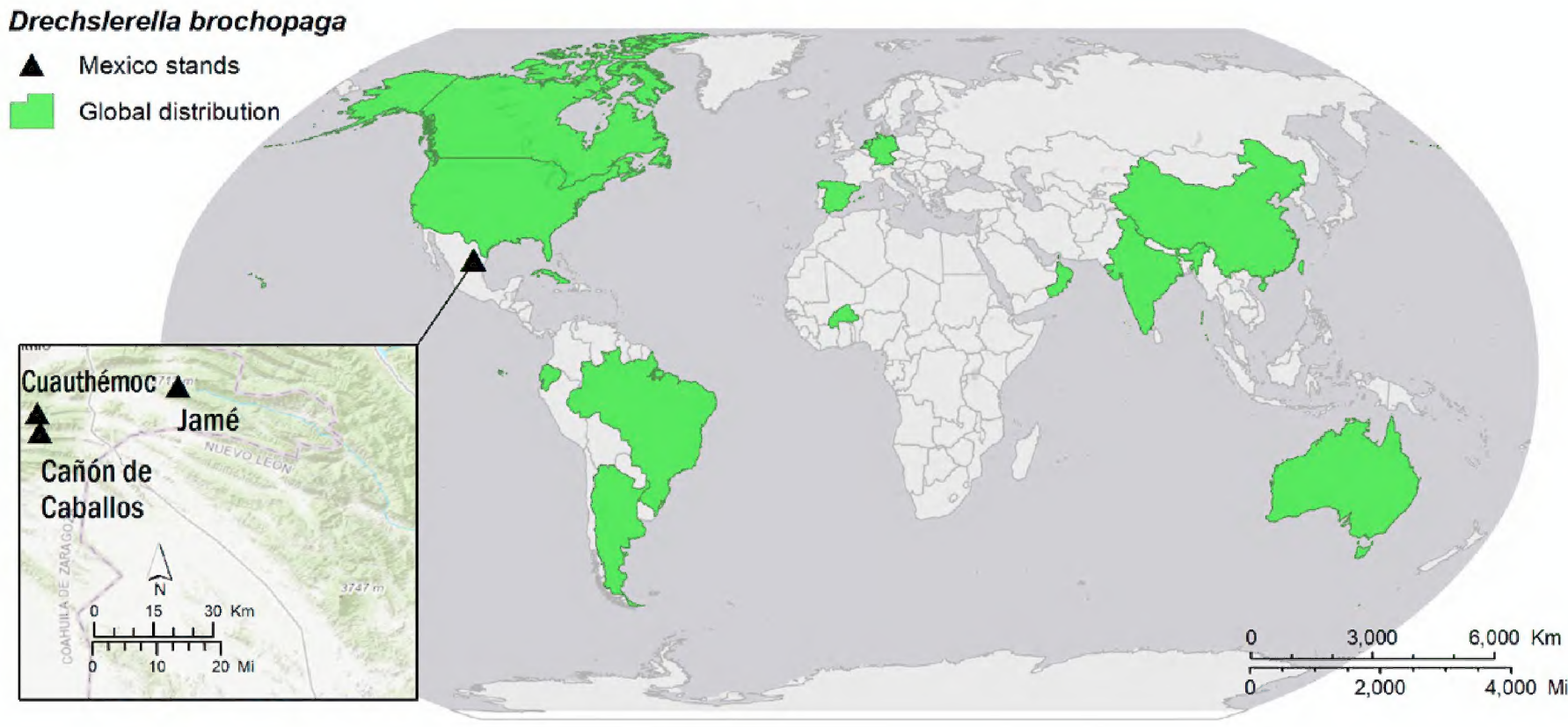

Figure 7. Historical global and new Mexican records of *Drechslerella brochopaga*. © 2021 ESRI, Here.

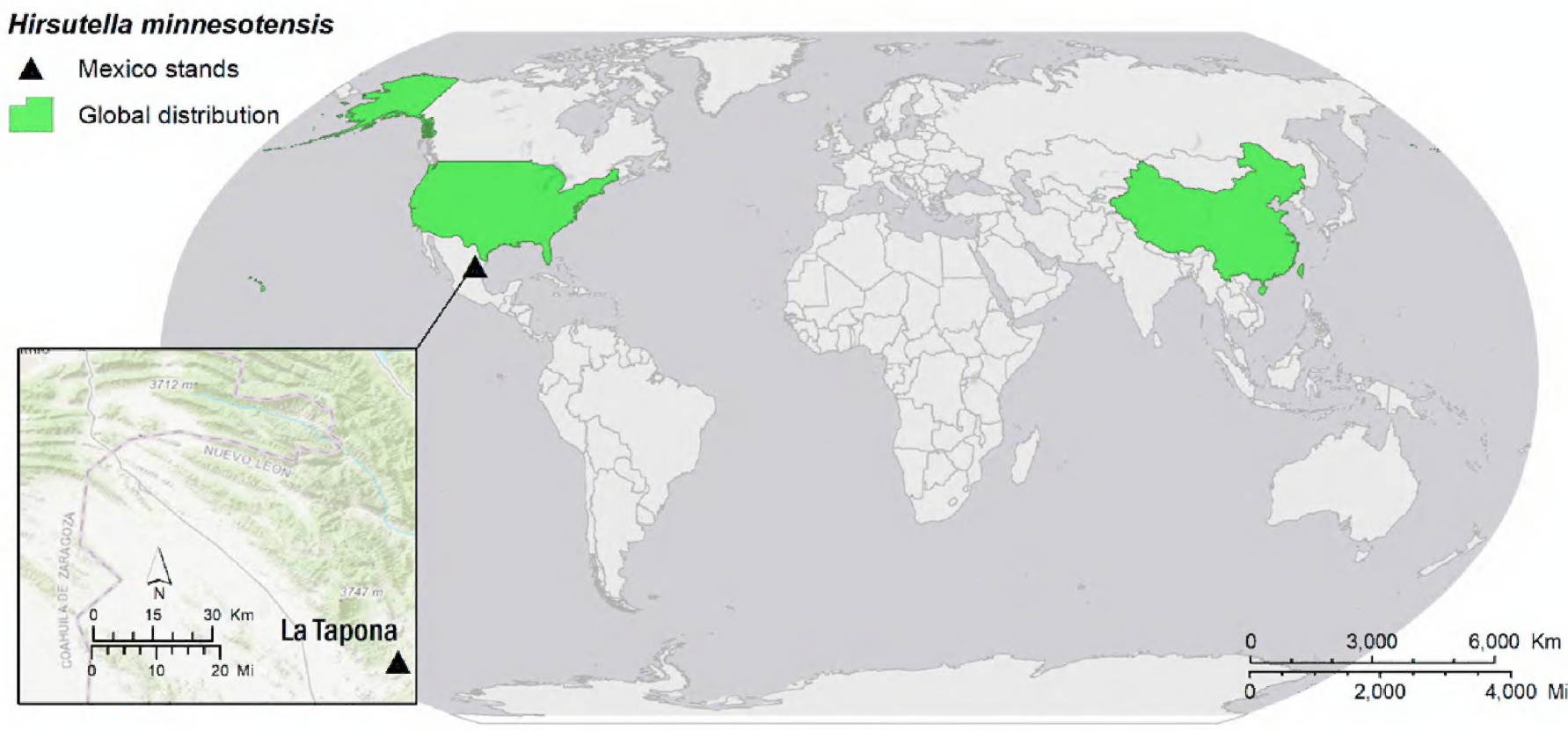

Figure 8. Historical global and new Mexican records of *Hirsutella minnesotensis*. © 2021 ESRI, Here.

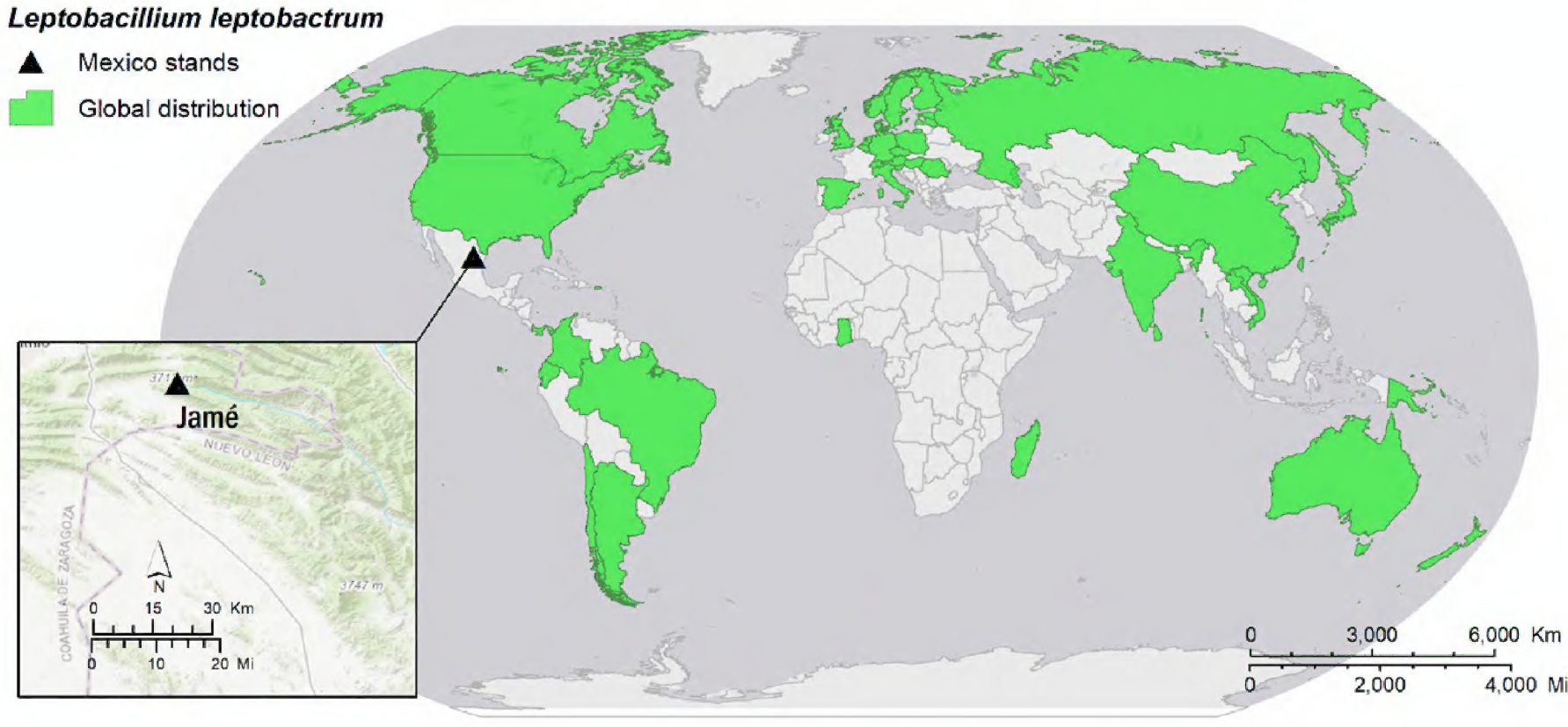

Figure 9. Historical global and new Mexican records of *Leptobacillium leptobactrum*. © 2021 ESRI, Here.

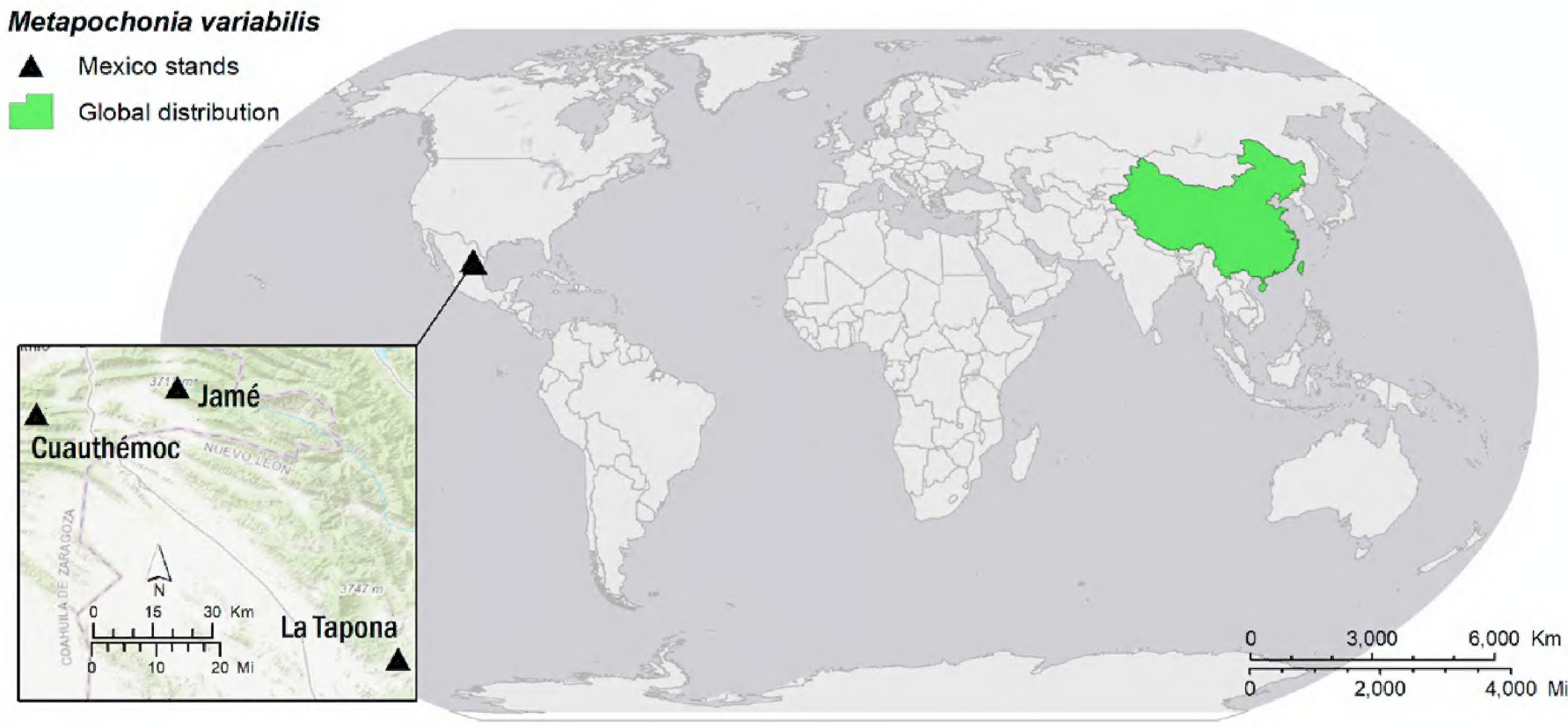

Figure 10. Historical global and new Mexican records of *Metapochonia variabilis*. © 2021 ESRI, Here.

est/roots sample/pooled DNA; Blast ID 645; GenBank MK412833.1 (ITS) – **Nuevo León** • La Tapona; 24° 43′39.90″N, 100°06′44.33″W; 2080 m alt.; 24.I.2015; same collector leg.; pine forest/pooled/roots/pooled DNA; Blast ID 645; GenBank MK412833.1 (ITS). Figure 10.

Identification. Obtained OTUs as described above were Blastn searched; 100% identity match with GenBank accession KU746684.1 (69 sequences found).

***Monacrosporium leptosporum* (Drechsler) A. Rubner**

New records. MEXICO – **Coahuila** • Arteaga, Jame; 25°23′00″N, 100°30′60″W; 2510 m alt.; 08.I.2015; R. Casique-Valdés leg.; oak-pine forest/roots sample/pooled DNA; Blast ID 1368; GenBank MK412835.1 (ITS) • Cuauhtémoc; 25°17′17″N, 100°55′06″W; 2423 m alt.; 12.I.2015; R. Casique-Valdés leg.; pine forest/roots sample/pooled DNA; Blast ID 1368; GenBank MK412835.1 (ITS) – **Nuevo León** • La Tapona; 24°43′39.90″N, 100°06′44.33″W; 2080 m alt.; 24.I.2015; same collector leg.; pine forest/pooled/roots/pooled DNA; Blast ID 1368; GenBank MK412835.1 (ITS). Figure 11.

Identification. Generated amplicons were sequenced (Illumina) using a MiSeq (2×300 bp) instrument. Filtered sequences were clustered to OTUs and Blastn searched; 96.2% identity match with GenBank accession AY773466.1 with 16 sequences.

Discussion

We report FPI associated with roots of an endemic Mexican pine species. The most speciose fungi were predaceous Orbiliales (eight species), which produce hyphal traps that capture nematodes (Treonis 2017). Nonetheless, common, very widespread species like *Arthrobotrys musiformis* Drechsler and *Arthrobotrys oligospora* Fresen. were not detected; the widely distributed *A. conoides* Drechsler was detected. The first two species mentioned are usually found at sites of disturbance, like agricultural fields, suggesting that they might be anthropophilic (Jansson and Llorca 2001) and possibly explain their absence from relatively pristine places, such as our sites. Among hypocralean entomopathogens, taxa from species complexes (morphologically indistinguishable species) in *Beauveria* and *Metarhizium* (Fisher et al. 2011; Rehner et al. 2011), as well as *Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha & Spatafora (= *Isaria farinosa*, *Paecilomyces farinosus*), are frequently isolated from temperate soils (Meyling and Eilenberg 2007; Masoudi et al. 2020). Our records of entomopathogens are very similar to those by Masoudi et al. (2020) for forests and reforested parkland, and presumably artificial grasslands in China, with the noteworthy absence in our study of the rhizospheric, endophytic PARBH clade. These are the well-known, common species of *Metarhizium*: *M. pingshaense* Chen & Guo, *M. anisopliae* (Metschnikoff) Sorokin, *M. robertsii* Bischoff, Rehner & Humber, *M. brunneum* Petch and *M. humberi* Luz, Rocha & Delalibera (St. Leger 2008; Fisher et al. 2011). These *Metarhizium* species are also widespread in agricultural and disturbed soils, but they are less frequently detected in undisturbed sites like natural forests (Vänninen et al. 2000; Sánchez-Peña et al. 2011; Guizar-Guzmán et al. 2013; Pérez-Gonzalez et al. 2014; Kepler et al. 2015). This might explain their absence from this analysis, as mentioned above for some species of *Arthrobotrys*.

Three taxa of *Beauveria* were recorded: *Beauveria bassiana* (Balsamo) Vuill. and *Beauveria pseudobassiana* Rehner & Humber (members of the *B. bassiana* complex) (Rehner et al. 2011) and *Beauveria felina*. The phylogenetic analysis using ITS sequences of Zhang et al. (2017) identified distinctively *B. bassiana*, *B. pseudobassiana* and *B. felina*. This is the second record of *B. pseudobassiana* in Mexico outside agricultural areas of central Mexico (near Mexico City) where it was associated with white grubs (Coleoptera, Scarabaeidae)

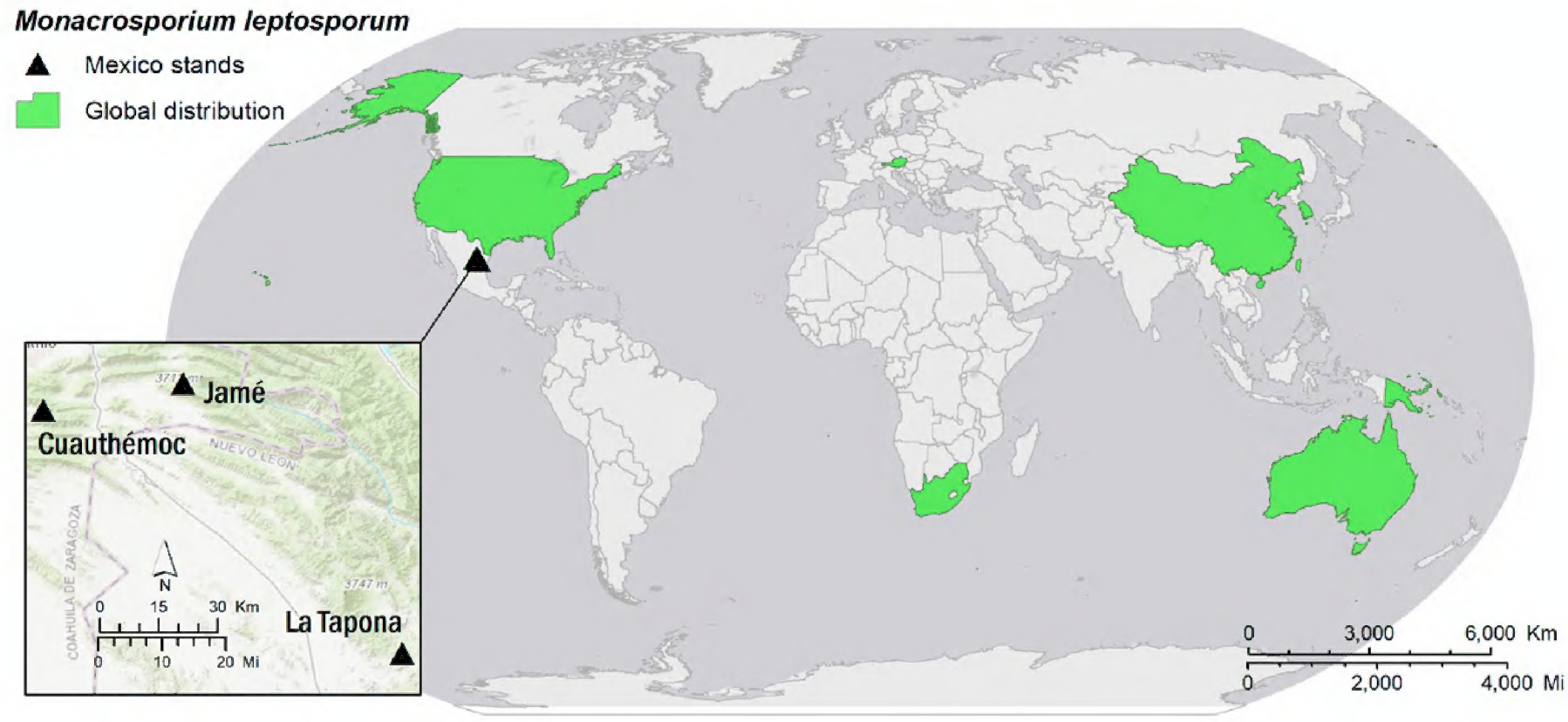

Figure 11. Historical global and new Mexican records of *Monacrosporium leptosporum*. © 2021 ESRI, Here.

(Carrillo-Benítez et al. 2013); it is also its first record in natural areas of the country. The finding of *B. pseudobassiana* associated with *Pinus* forests, where white grubs are not abundant, and widely separated from central Mexico, suggests wide distribution and adaptability to a variety of environments. We also provide the first report of *Beauveria felina* in Mexico. This fungus is not commonly found as an entomopathogen; however, it was reported recently causing epizootics on Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith), on maize in India (Ramanujam et al. 2021). The fungus has been synonymized with *Amphicorda felina* (DC) Fr. The taxonomic characterization of isolates identified as *B. felina* should be critically reviewed, since it appears to be a *nomen confusum* or a case of homonymy; for example, Zhang et al. (2017) appear to suggest simple phialidic conidiogenous cells in *Amphicorda*, while the Mycobank database for *B. felina* suggests polyblastic conidiogenous cells in this species. The variability of this character should be clarified. The phylogenetic analysis by Zhang et al. (2017) placed several isolates of *Amphicorda* and *B. felina* in the Hypocreales, possibly in the Ophiocordycipitaceae.

We detected *Cordyceps farinosa* in these forests. This was the most common fungal pathogen attacking the hemlock wholly adelgid, *Adelges tsugae* (Annand) (Hemiptera, Adelgidae) in temperate hemlock forests in China, and the second most abundant pathogen in similar forests in the northeastern USA (Reid et al. 2010). It is also reported from tropical primary forests in Mexico (Sanchez-Peña 1990).

The finding of the entomopathogenic fungus *Zoophthora radicans* from roots, most probably from the rhizoplane, is noteworthy. This constitutes the first report of its detection from environmental samples. This widespread, polyphagous fungus is a significant biological control agent of different insect pests such as Diamondback Moth (Ullyett 1947; Guzmán-Franco et al. 2008), Potato Leafhopper (Galaini-Wraight et al. 1991) Painted (*Bagrada*) Bug, and Potato-Tomato Psyllid (Torres-Acosta et al. 2016). With the exception of species of *Conidiobolus*, FPI in the Entomophthorales are difficult and fastidious to cultivate, isolate or detect in the environment from non-insect substrata (Jensen et al. 2007; Fournier et al. 2008).

We found several OTUs with high coverage and consistent (82–96%) identity with taxa in the same families of FPI, like Orbiliaceae (i.e., *Dactylella* spp., *Hyalorbilia oviparasitica* (G.R. Stirling & Mankau) E. Weber & Bara, *Monacrosporium leptosporum*, and *Orbilia* spp.), Cordycipitaceae (i.e., *Lecanicillium* and *Simplicillium*), and uncultured fungi. These OTUs are likely non-barcoded species in these taxa.

In this work we report low-abundance (doubletons; no singletons found) sequences found in the community of invertebrate-pathogenic fungi. Although some authors have suggested that singletons and doubletons are potential artifacts, the sequences reported herein have a high percentage of identity (97% cutoff) and coverage. The reporting of these low-abundance sequences is not likely to modify trends in ecological and community studies (Brown et al. 2015). In fact, these rare OTUs can imply the existence of a "rare biosphere" that includes a very high number of phylotypes with low abundance (the long tail of rank abundance curves) that should be considered as indicators of true diversity (Huse et al. 2010; Brown et al. 2015). Indiscriminate discarding of rare sequences can lead to failed population assessment and underestimation of biodiversity (Brown et al. 2015). These low-abundance sequences portray rare OTUs that define complex microbial communities (Huse et al. 2010).

In conclusion, we detected a large and diverse array of nematophagous and entomopathogenic fungi in the *P. greggii* stands sampled. We found FPI usually found in natural habitats like *Tolypocladium* (= *Elaphocordyceps*) and *Cordyceps farinosa*, and FPI widespread in agricultural and disturbed areas: *B. bassiana*, *B. pseudobassiana*,

P. lilacinum, and *Z. radicans*, suggesting broad ecological adaptations in these organisms. The first detection of the entomophthoralean *Z. radicans* from environmental samples is remarkable. This work reinforces that widespread entomopathogenic species of the PARBH clade of *Metarhizium* and some nematophagous *Arthrobotrys* species appear to be anthropophilic fungi that thrive in agricultural and disturbed sites but are seemingly absent from more pristine forest sites. The remoteness of the forest stands sampled could provide an opportunity to test hypotheses on the effect of disturbances on FPI populations. Efforts on biological control of pests might benefit from novel natural enemies collected at relatively undisturbed sites like these Mexican forests.

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Authors' Contributions

Conceptualization: RCV, SA, SRSP. Data analysis: SA. Formal analysis: RCV, SA, SRSP. Methodology: RCV and SRSP. Software: SA and FGG. Visualization: FGG. Original draft writing, review and editing: RCV, SA, FGG and SRSP.

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